

FIG.1

MOLECULES INVOLVING, etc. REPLACEMENT SHEET 2/7 UPPER PRIMER 1044 SEQ ID NO:1 LOWER PRIMER LABEL AMPLIFICATION OF TARGET NUCLEIC ACID IN THE PRESENCE OF dGTP, dATP, dUTP, AND dCTP AND ^{32}P LABELLED LOWER PRIMER. 952 - Treatment with exonuclease I AND SHRIMP ALKALINE PHOSPHATASE. - TREATMENT WITH UDG. -TREATMENT WITH NaOH AT 95°C 1020 1044 (ONLY LABELLED FRAGMENTS SHOWN HERE) SEQ ID NO:4 REMOVAL OF 3' PHOSPHATE BY TREATMENT WITH T4 PNK SEQ ID NO:5 (ONLY LABELLED FRAGMENTS SHOWN HERE) LINEAR AMPLIFICATION OF DNA (952 TO 1044) USING LABELLED UPSTREAM FRAGMENT FOLLOWED BY ANALYSIS ON DENATURING POLYACRYLAMIDE GEL, FOLLOWED BY AUTORADIOGRAPHY. 952 1044 93MER, EXTENDED UPSTREAM FRAGMENT SEQ ID NO:6 952 = 1044 SEQ ID NO:1

Docket No.: 1377-0156P

App No.: 09/673,739 Conf. #3757

Inventor: Thomas V. MCCARTHY et al.

Title: A METHOD FOR THE CHARACTERISATION ON NUCLEIC ACID

FIG.2

TEMPLATE DNA

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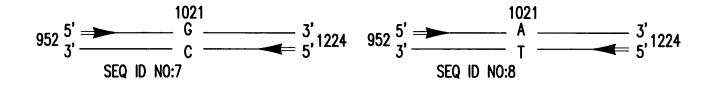
MOLECULES INVOLVING, etc.

REPLACEMENT SHEET

3/7

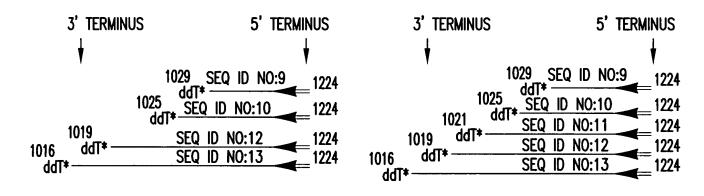
– AMPLIFICATION OF NORMAL AND MUTANT TARGET NUCLEIC ACID IN THE PRESENCE OF dGTP, dATP, dCTP AND 1/20 RATIO OF dUTP TO dTTP.

Docket No.: 1377-0156P



- TREATMENT WITH EXONUCLEASE I AND SHRIMP ALKALINE PHOSPHATE.
- Treatment with UDG.
- TREATMENT WITH NaOH AT 95°C
- DNA IS PRECIPITATED.
- TREATMENT WITH T4 PNK

EXTENSION OF THE UPSTREAM FRAGMENTS GENERATED ABOVE IN THE PRESENCE OF 33P-LABELLED ddTTP* AND UNLABELLED ddGTP, ddATP, AND ddCTP.



ONLY SOME FRAGMENTS CORRESPONDING TO CLEAVAGE AT U INCORPORATION SITES SURROUNDING THE MUTATION SITE ARE SHOWN HERE.

DETECTION OF EXTENDED LABELLED FRAGMENTS BY PAGE AND AUTORADIOGRAPHY

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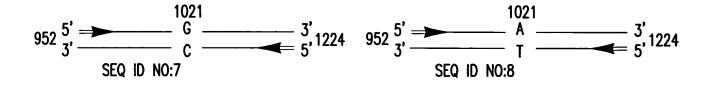
REPLACEMENT SHEET

Sheet 4 of 7

Docket No.: 1377-0156P

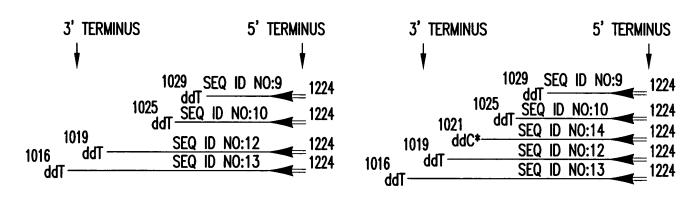
4/7

- AMPLIFICATION OF NORMAL AND MUTANT TARGET NUCLEIC ACID IN THE PRESENCE OF dGTP, datp, dctp and 1/20 ratio of dutp to dttp.



- TREATMENT WITH EXONUCLEASE I AND SHRIMP ALKALINE PHOSPHATE.
- TREATMENT WITH UDG.
- TREATMENT WITH NaOH AT 95°C
- DNA IS PRECIPITATED.
- Treatment with T4 PNK

EXTENSION OF THE UPSTREAM FRAGMENTS GENERATED ABOVE IN THE PRESENCE OF ³³P-labelled ddctp* and unlabelled ddgtp, ddatp, and ddttp.



ONLY SOME FRAGMENTS CORRESPONDING TO CLEAVAGE AT U INCORPORATION SITES SURROUNDING THE MUTATION SITE ARE SHOWN HERE.

1021 SEQ ID NO:14 1224

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MOLECULES INVOLVING, etc. REPLACEMENT SHEET

Sheet 5 of 7

Docket No.: 1377-0156P

5/7

6390 UPPER PRIMER A SEQ ID NO:15 6443

5' AACTTGTGGTAGTTGGAGCTGGTGGCGTAGGCAAGAGTGCCTTGACGATACAGC 3'
3' TTGAACACCATCAACCTCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5'

T LOWER PRIMER

AMPLIFICATION OF TARGET NUCLEIC ACID IN THE PRESENCE OF dGTP, datp, dutp, and dctp.

SEQ ID NO:16

AMPLIFIED MUTANT ALLELE

- 5' AACTTGTGGTAGTTGGAGCTGGUGGCGUAGGCAAGAGUGCCUUGACGAUACAGC 3'
- 3' UUGAACACCAUCAACCUCGACUACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' SEQ ID NO:17

SEQ ID NO:18

AMPLIFIED MUTANT ALLELE

- 5' AACTTGTGGTAGTTGGAGCTGAUGGCGUAGGCAAGAGUGCCUUGACGAUACAGC 3'
- 3' UUGAACACCAUCAACCUCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' SEQ ID NO:19
 - TREATMENT WITH EXONUCLEASE I
 AND SHRIMP ALKALINE PHOSPHATASE.
 - TREATMENT WITH UDG.
 - TREATMENT WITH ENDO IV.

SEQ ID NO:20 NORMAL UPSTREAM FRAGMENT
3' CGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5'

SEQ ID NO:21 MUTANT UPSTREAM FRAGMENT
3' ACCGCATCCGTTCTCACGGAACTGCTATGTCG 5'

6/7 REVERSE PRIMER SEQ ID NO:23 5' GCTGTAAACGACGCCAGTTTCAT 3' SYNTHETIC TEMPLATE NO.1 SEQ ID NO:22 5' GCTGTAAACGACGGCCAGTTTCATGCAGGGCTGGAGTCGTAGGCAAGAGTGCCTTGACGATACAGC 3' X3' CGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' NORMAL UPSTREAM FRAGMENT PCR AMPLIFICATION IN PRESENCE OF α^{32} PdCTP **SEQ ID NO:20** FOLLOWED BY DENATURING PAGE **SEQ ID NO:24** 3' CGACATTTGCTGCCGGTCAAAGTACGTCCCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' * ** * ** В REVERSE PRIMER SEQ ID NO:23 5' GCTGTAAACGACGGCCAGTTTCAT 3' SYNTHETIC TEMPLATE NO.2 SEQ ID NO:25 5' GCTGTAAACGACGGCCAGTTTCATGCAGGATCCATGGCGTAGGCAAGAGTGCCTTGACGATACCGC 3' XXXXX 3' CGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' NORMAL UPSTREAM FRAGMENT **SEQ ID NO:20** PCR AMPLIFICATION IN PRESENCE OF α^{32} PdCTP FOLLOWED BY DENATURING PAGE X C REVERSE PRIMER SEQ ID NO:23 5' GCTGTAAACGACGGCCAGTTTCAT 3' SYNTHETIC TEMPLATE NO.2 SEQ ID NO:25 5' GCTGTAAACGACGGCCAGTTTCATGCAGGATCCATGGCGTAGGCAAGAGTGCCTTGACGATACAGC 3' 3' ACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' MUTANT UPSTREAM FRAGMENT PCR AMPLIFICATION IN PRESENCE OF α^{32} PdCTP SEQ ID NO:21 FOLLOWED BY DENATURING PAGE **SEQ ID NO:26** 3' CGACATTTGCTGCCGGTCAAAGTACGTCCTAGGTACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' * * * * * * * * * 66MER D REVERSE PRIMER **SEQ ID NO:23** 5' GCTGTAAACGACGGCCAGTTTCAT 3' SYNTHETIC TEMPLATE NO.1 **SEQ ID N0:22** GCTGTAAACGACGGCCAGTTTCATGCAGGGCTGGAGTCGTAGGCAAGAGTGCCTTGACGATACAGC 3' X X 3' ACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' MUTANT UPSTREAM FRAGMENT PCR AMPLIFICATION IN PRESENCE OF $\alpha^{\ensuremath{32}\xspace}\xspace{\text{PdCTP}}$ **SEQ ID NO:21** FOLLOWED BY DENATURING PAGE MISMATCHES DENOTED BY X X 32 P Label Denoted by *

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MOLECULES INVOLVING, etc. REPLACEMENT SHEET **SEQ ID NO:27** Α TEMPLATE OLIGO 1 GGTAGTTGGAGCTGGTGGCG SEQ ID NO:20 CGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG CCATCAACCT* 3' REPORTER 5' NORMAL UPSTREAM FRAGMENT 0LIG0 1 LIGATION REACTION FOLLOWED BY **SEQ ID NO:28** DENATURING PAGE **SEQ ID NO:29** CCATCAACCTCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' 47MER SEQ ID NO:27 TEMPLATE OLIGO 1 **GGTAGTTGGAGCTGGTGGCG** SEQ ID NO:20 ĊĠĂĊĊĂĊĊĠĊATCCGTTCTCACGGAACTGCTATGTCG AACCTCGACC* 3'RFPORTER 5' NORMAL UPSTREAM FRAGMENT 0LIG0 2 LIGATION REACTION FOLLOWED BY **SEQ ID NO:30** DENATURING PAGE AACCTCGACC* SEQ ID NO:30 10MER **SEQ ID NO:31** C TEMPLATE OLIGO 2 TTGGAGCTGGTGGCGTAGGC SEQ ID NO:21 .///////// AACCTCGACC* ACCGCATCCGTTCTCACGGAACTGCTATGTCG MUTANT UPSTREAM FRAGMENT 3 REPORTER 5 0LIG0 2 LIGATION REACTION FOLLOWED BY **SEQ ID NO:30** DENATURING PAGE SEQ ID NO:32 AACCTCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' 42MER **SEQ ID NO:31** D TEMPLATE OLIGO 2 TTGGAGCTGGTGGCGTAGGC SEQ ID NO:21 CCATCAACCT* ACCGCATCCGTTCTCACGGAACTGCTATGTCG 3' REPORTER 5' MUTANT UPSTREAM FRAGMENT 0LIG0 1 32P LABEL DENOTED BY * LIGATION REACTION FOLLOWED BY **SEQ ID NO:28** DENATURING PAGE >> DENOTES BASEPAIRING **SEQ ID NO:28** CCATCAACCT*

Docket No.: 1377-0156P

FIG.7

App No.: 09/673,739 Conf. #3757

3'

5

10MER

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